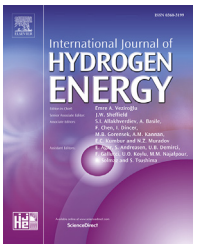
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2017) 1–12

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he



Optimization of biohydrogen production by the novel psychrophilic strain N92 collected from the Antarctica

Sergio Cisneros de la Cueva, Cecilia L. Alvarez Guzmán, Víctor E. Balderas Hernández, Antonio De León Rodríguez *

División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, A.C. Camino a La Presa San José 2055, Col. Lomas 4a Sección, San Luis Potosí, SLP, C.P. 78216, Mexico

ARTICLE INFO

Article history:
Received 5 September 2017
Received in revised form
27 November 2017
Accepted 29 November 2017
Available online xxx

Keywords:
Biohydrogen
Central composite design
Dark fermentation
Psychrophilic bacteria
Response surface methodology

ABSTRACT

In this study, the response surface methodology (RSM) with central composite design (CCD) was employed to improve the hydrogen production by the psychrophilic N92 strain (EU636058) isolated from Antarctica, which is closely related to *Pseudorhodobacter* sp. (KT163920). The influence of operational conditions such as temperature (4.7–55.2 °C), initial pH (3.44–10.16), and initial glucose concentration (4.7–55.23 g/dm^3), as well as the initial concentrations of $(NH_4)_2SO_4$ (0.05–3.98 g/dm^3), $FeSO_4$ (0.02–1.33 g/dm^3) and $NaHCO_3$ (0.02–3.95 g/dm^3) was evaluated. The linear effect of glucose concentration, along with the quadratic effect of all the six factors were the most significant terms affecting the biohydrogen yield by N92 strain. The optimum conditions for the maximum hydrogen yield of 1.7 mol H_2/mol glucose were initial pH of 6.86, glucose concentration of 28.4 g/dm^3, temperature 29 °C and initial concentration of $(NH_4)_2SO_4$, $FeSO_4$ and $NaHCO_3$ of 0.53, 1.55 and 1.64 g/dm^3 respectively. Analysis of the metabolites produced under the optimum conditions showed that the most abundant were acetic acid (0.8 g/dm^3), butyric acid (0.7 g/dm^3) and ethanol (2.1 g/dm^3). We suggest that the bioprocess established in this study using the strain N92 could be an alternative for hydrogen production with the advantages of constituting low energy costs in fermentation.

Introduction

Hydrogen is considered as an attractive future energy carrier and it is preferred over biogas or methane because hydrogen is not chemically bound to carbon, and therefore, its burning does not contribute to greenhouse gases or acid rain [1]. There are several approaches to produce hydrogen such as steam reforming of natural gas, coal gasification and water electrolysis, as well as novel chemical processes like the hydrolysis of hydrides with steam, which combines both hydrogen production and storage in one step [2–9]. On the other hand, there are the biological methods which mostly operate at ambient temperatures and pressures [8,10]. These approaches mainly include photosynthetic and dark fermentative hydrogen production. However, dark fermentation has advantages over other processes because of its ability to

* *Corresponding author.*
E-mail addresses: aleonr@ipicyt.edu.mx, aleonr@me.com (A. De León Rodríguez).

https://doi.org/10.1016/j.ijhydene.2017.11.164
0360-3199/

continuously produce hydrogen from a number of renewable feedstock [11]. During the dark fermentative process, when glucose is used as model substrate under anoxic conditions, bacteria convert glucose to pyruvate through glycolytic pathways producing adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and the reduced form of nicotinamide adenine dinucleotide (NADH). Pyruvate is further oxidized to acetyl coenzyme A (acetyl-CoA), carbon dioxide (CO_2) and H_2 by pyruvate ferredoxin oxidoreductase and hydrogenase. Depending on the type of microorganism and environmental conditions, pyruvate may also be converted to acetyl-CoA and formate, which may be further converted into H_2 and CO_2. Also, acetyl-CoA might be converted to acetate and ethanol [12]. This process is complex and influenced by many factors such as inoculum, substrate, nitrogen, phosphate, metal ion, temperature and pH [13]. The effect of these factors has been widely studied, however, most of the fermentative hydrogen production processes are focused on the use of mesophilic and thermophilic microorganisms and there are few reports available addressing psychrophilic bacteria [14–16]. The use of psychrophilic hydrogen producing microorganisms could be an economical advantage due to its operation temperatures. These microorganisms have high enzymatic activities and catalytic efficiencies in the 0–20 °C temperature range in which homologous mesophilic enzymes are less active, and allow to renounce on expensive heating/cooling systems, thus constituting a considerable progress towards the saving of energy [17]. Therefore, the aim of this experimental work was the production of hydrogen using a newly psychrophilic N92 strain isolated from Antarctica [18]. Since there is insufficient information about the operational conditions for psychrophilic hydrogen production, we have applied the response surface methodology to set the optimum operating conditions and media composition to reach the maximum hydrogen production. In this context, temperature, pH and substrate concentration are important factors influencing the activity of bacteria towards hydrogen production. Moreover, temperature is a key factor since it might alter process efficiency, hydrogen production activity, and liquid product distribution by influencing the bacterial enzymatic activity. Kumari and Das [19] reported that an initial pH in an inadequate range affects the activity of the hydrogenase enzymes as well as an inadequate initial substrate concentration affects metabolic pathways decreasing the production of biohydrogen. On the other hand, at the cellular level, some elements have certain effects on the activity of hydrogen-producing bacteria, particularly the concentrations of nitrogen and iron, essential nutrients for hydrogen production, as well as buffer supplementation [13]. Iron is an essential component of ferredoxin and hydrogenase that catalyze the reduction of H^+ to H_2. The Fe–S bindings are responsible for the transport of electrons in specific proteins that participate on the pyruvate oxidation to acetyl-CoA, CO_2 and H_2. It has been reported that the *in vivo* activity of the hydrogenase decreases with iron depletion, suggesting that the presence of iron in the fermentation medium is required to preserve the bacteria and prevent the loss of its characteristics. Moreover, nitrogen is one of the essential nutrients needed for the growth of hydrogen-producing bacteria. A source of nitrogen is demanded for the syntheses of proteins, nucleic acids, and enzymes. Among the sources of nitrogen, ammonia has been applied in hydrogen production by dark fermentation, it is a cheap inorganic nitrogen source compared to other organic nitrogen sources and it has been shown that in an appropriate concentration range, ammonia nitrogen is beneficial to fermentative hydrogen production, while at a much higher concentration could have an inhibitory effect for it may change the intracellular pH of hydrogen-producing bacteria, increase the maintenance energy requirement or inhibit specific enzymes related to fermentative hydrogen production [20]. Low or high concentration of these nutrients may cause low hydrogen yields. Therefore, in this work the effects of these operational factors (temperature, pH and substrate concentration) and mineral nutrient concentration (ammonia, carbonate and ferrous ion) on hydrogen production were studied using two central composite designs to obtain optimum hydrogen production conditions by the psychrophilic N92 strain.

Material and methods

Microorganism and growth media

In this work, the strain N92 (EU636058) highly related to *Pseudorhodobacter* sp. (KT163920) according to NCBI blast was used. It was isolated from samples of glacier sediment from Antarctica [18]. The strain was grown in YPG agar plates in g/dm^3 (2.75 of Bacto-tryptone, 0.25 of yeast extract, 25 of glucose and 15 of Bacto-agar) and maintained at 4 °C [15].

Experiment designs

The first central composite design with two center points was implemented to optimize the temperature, initial pH and initial glucose concentration to maximize biohydrogen yield by batch cultures fermentations of N92 strain (Table 1) [21].

A second order polynomial mathematical model (Eq. (1)) was proposed to describe the effects of several factors on the response based on experimental results.

$$Y_i = \beta_0 + \sum_{i}^{\beta} x_i + \sum_{ii}^{\beta} x_i^2 + \sum_{ij}^{\beta} x_i x_j \quad (1)$$

where Y_i is the corresponding response, x_i and x_j are the independent variables, β_0 is the model intercept, βi are the linear coefficients, βii are the squared coefficients and βij are the interaction coefficients [13]. In addition, the analysis of variance (ANOVA) was used to obtain the relationship between independent variables and the response, as well as to describe the effects of several factors on the response based on the

Table 1 – Independent variables and levels used in the experimental design to optimize the operational factors.

Independent variables	Levels		
	−1	0	1
X_1-Temperature (°C)	15	30	45
X_2-pH	4.8	6.8	8.8
X_3-Glucose concentration (g/dm^3)	15	30	45

Please cite this article in press as: Cisneros de la Cueva S, et al., Optimization of biohydrogen production by the novel psychrophilic strain N92 collected from the Antarctica, International Journal of Hydrogen Energy (2017), https://doi.org/10.1016/j.ijhydene.2017.11.164